EXHIBIT 1

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Fetal and adult human oligodendrocyte progenitor cell isolates myelinate the congenitally dysmyelinated brain

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Both late-gestation and adult human forebrain both contain large numbers of oligodendrocyte progenitor cells (OPCs). These cells may be identified by their A2B5$^+$PSA-NCAM$^-$ phenotype (positive for the early oligodendrocyte marker A2B5 and negative for the polysialylated neural cell adhesion molecule). We used dual-color fluorescence-activated cell sorting (FACS) to extract OPCs from 21- to 23-week-old fetal human forebrain, and A2B5 selection to extract these cells from adult white matter. When xenografted to the forebrains of newborn shiverer mice, fetal OPCs dispersed throughout the white matter and developed into oligodendrocytes and astrocytes. By 12 weeks, the host brains showed extensive myelin production, compaction and axonal myelination. Isolates of OPCs derived from adult human white matter also myelinated shiverer mouse brain, but much more rapidly than their fetal counterparts, achieving widespread and dense myelin basic protein (MBP) expression by 4 weeks after grafting. Adult OPCs generated oligodendrocytes more efficiently than fetal OPCs, and ensheathed more host axons per donor cell than fetal cells. Both fetal and adult OPCS phenotypes mediated the extensive and robust myelination of congenitally dysmyelinated host brain, although their differences suggested their use for different disease targets.

A broad range of pediatric leukodystrophies and storage diseases manifest with myelin failure or loss. Recent studies have focused on the use of transplanted oligodendrocytes or their progenitors to treat congenital myelin diseases. The myelinogenic potential of implanted brain cells was first noted in the shiverer mouse[1,2]. Shiverer is an autosomal recessive mutation; *shi/shi* homozygotes fail to develop MBP or compact myelin and die by 20–22 weeks. Transplanted fetal brain cells[3–6], primary[7] and immortalized[8] neural progenitors, and enriched glial progenitor cells[9] can all myelinate shiverer axons, albeit typically with low efficiency. Similarly, rodent subventricular zone progenitors can engraft another dysmyelinated mutant, the myelin-deficient rat, after perinatal administration[10,11]. Indeed, all of these studies suggest the feasibility of myelinating congenitally dysmyelinated brain, even though none of the cell sources used did so efficiently.

On this basis, we asked whether highly enriched populations of OPCs directly isolated from the human brain might be used as more effective substrates for cell-based therapy of congenital dysmyelination. Specifically, we postulated that human OPCs, whether derived from the fetal brain during its period of maximum oligoneogenesis, or from the adult subcortical white matter[12,13], could mediate large-scale myelination of a congenitally dysmyelinated host. We report here that both fetal and adult human OPCs, highly enriched by surface antigen–based FACS, were capable of widespread and high-efficiency myelination of the shiverer mouse brain after perinatal xenograft. We also report significant differences in the behavior of fetal and adult-derived OPCs, which suggests that they may be useful in treating different specific disease targets.

Cells dissociated from the late second-trimester human ventricular zone (21–23 weeks gestation) were first magnetically sorted to isolate A2B5$^+$ cells[13–16], including oligodendrocytic and neuronal progenitor cells. Because PSA-NCAM is expressed by immature neurons at this stage of development[17], we then used FACS to deplete PSA-NCAM$^+$ neurons from the larger A2B5$^+$ cell population. This yielded a subpopulation of A2B5$^+$PSA-NCAM$^-$ cells, which defined our oligodendrocyte progenitor pool. Two-color FACS showed that the A2B5$^+$PSA-NCAM$^-$ fraction constituted 15.4 ± 4.8% of the cells in samples from the 21- to 23-week ventricular zone (n = 5; **Supplementary Figure 1** online). Of these A2B5$^+$PSA-NCAM$^-$ cells, 76.1 ± 0.5% expressed oligodendrocytic O4 by 1 week after FACS, whereas only 7.5 ± 0.3% expressed astrocytic glial fibrillary acidic protein (GFAP) and only 2.0 ± 1.3% expressed neuronal β-III tubulin. These data support the glial restriction and oligodendrocytic bias of sorted A2B5$^+$PSA-NCAM$^-$ cells. Because we achieved higher net yields with immunomagnetic separation of A2B5$^+$ cells followed by FACS depletion of NCAM$^+$ cells, compared with two-color FACS, we used this technique for progenitor isolation.

Homozygous *shi/shi* mice were injected intracallosally with fetal progenitor cell isolates on either their day of birth (P0) or on postnatal day 1 (P1), and later killed at 4, 8, 12 or 16 weeks of age. None of the animals were immunosuppressed; we relied on perinatal tolerization to ensure graft acceptance[18,19]. The injections resulted in substantial engraftment, defined as ≥100 cells per coronal section at three rostrocaudal levels sampled >100 mm apart, in 34 of the 44 neonatal mice injected for this study (25 of 33 injected with fetal human OPC, and 9 of the 11 injected with adult-derived OPCs). By 12 weeks of age, the recipients showed donor engraftment throughout the callosum and capsular and commissural

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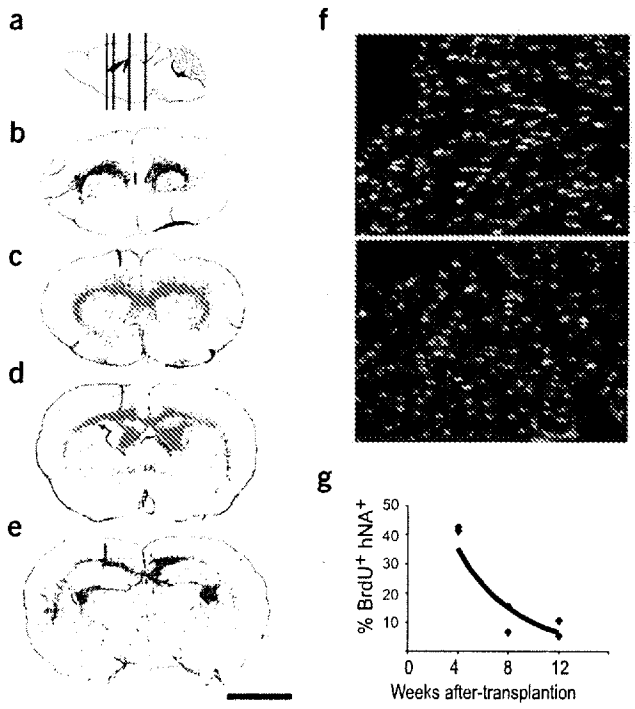

Figure 1 Fetal human OPCs disperse rapidly to infiltrate the forebrain. (**a–e**) Human cells were localized by immunostaining for hNA. Low-power fluorescence images of coronal section of forebrain(**b–e**) were collected at representative anteroposterior levels as indicated in schematic (**a**; ref.25) Engrafted cells are shown in red (**b–e**). (**f**) Immunofluorescent detection of BrdU (green) and hNA (red) 4 (top) and 12 (bottom) weeks after xenograft of human OPCs into shiverer mice. Arrows indicate mitotic human OPCs ($BrdU^+hNA^+$). (**g**) Regression plot of mitotically active donor cells as a function of time after perinatal implant. Rate of BrdU incorporation declined according to the exponential regression $y = 83.4e^{-0.22x}$, with correlation coefficient $r = -0.87$ ($P = 0.012$). Scale bar, 3 mm (**b–e**) or 50 μm (**f**).

white matter, extending caudally to the basis pontis (**Fig. 1a–e**). During this time, cell division among the engrafted progenitors, though initially high at 4 weeks, fell to relatively low and stable levels by 8 and 12 weeks (Fig. 1f,g). The fraction of human donor cells that incorporated BrdU during the 48 h before mice were killed dropped from 42 ± 6.1% at 4 weeks to 8.2 ± 2.4% at 12 weeks.

During this same period, many of the fetal progenitors matured into myelinogenic oligodendrocytes, as indicated by their expression of MBP. At 4 weeks, no MBP was detectable in 10 of 11 animals, despite widespread cell dispersion; scattered MBP^+ cells were noted in one mouse. At 8 weeks, patchy foci of MBP expression were noted in four of seven mice, and by 12 weeks, widespread MBP expression was noted throughout the forebrain white matter tracts in five of seven mice. By this time, the engrafted mice typically expressed MBP throughout the entire corpus callosum, as well as throughout the fimbria and internal capsules (**Fig. 2a–d**). Because shiverer mice express only the first exon of the *Mbp* gene[2], and hence have no immunodetectable MBP, any MBP detected in these recipients was necessarily donor-derived[8]. In addition, optical sectioning confirmed that the MBP^+ cells were of human origin, in that each MBP^+ profile was associated with a human nuclear antigen $(hNA)^+$ soma (**Fig. 2c,e–h**).

We next asked whether donor-derived myelin effectively wrapped host axons. We used confocal imaging and electron microscopy to assess axonal ensheathment and myelin compaction, respectively. Confocal analysis was first done on the brains of three shiverer mice that were implanted on P1 with 100,000 fetal human OPCs each, and sacrificed at 12 weeks. Foci of dense MBP expression were assessed by confocal imaging, after immunolabeling for hNA and neurofilament (NF) protein to detect donor-derived cells and host shiverer axons, respectively. We found

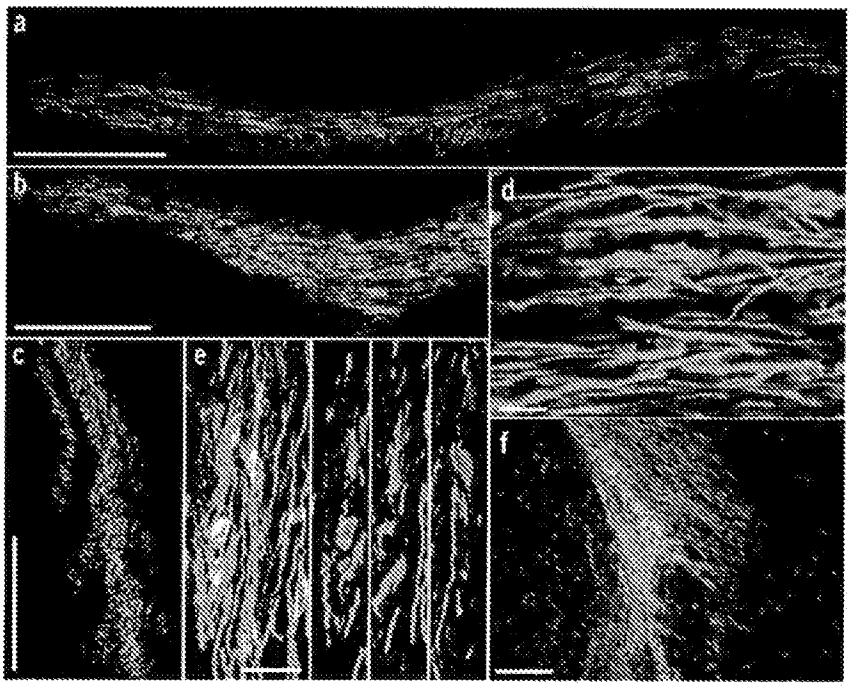

Figure 2 Engrafted human OPCs myelinate an extensive region of the forebrain. (**a,b**) MBP expression (green) by sorted human fetal OPCs implanted into homozygous shiverer mice. Large regions of the corpus callosum were myelinated by 12 weeks. **a** and **b** are two different mice. (**c**) Human OPCs migrated to and myelinated fibers throughout the dorsoventral extents of the internal capsules, resulting in widespread forebrain remyelination after a single perinatal injection. (**d**) MBP expression (green) in engrafted shiverer mouse callosum 3 months after perinatal xenograft was associated with human hNA^+ donor cells (red). (**e**) Confocal optical sections of implanted shiverer mouse callosum, with hNA^+ donor cells (red) surrounded by MBP (green). Human cells (arrows) were found within meshwork of MBP^+ fibers. Right three images, taken 1 μm apart, were merged to form left image. (**f**) Striatocallosal border of shiverer mouse brain, 3 months after perinatal engraftment with human fetal OPCs (blue). Donor-derived MBP^+ oligodendrocytes and myelin (red) are evident in the corpus callosum, while donor-derived $GFAP^+$ astrocytes (green) predominate on the striatal side. Scale bar, 1 mm (**a–c**), 100 μm (**d**), 20 μm (**e**) or 200 μm (**f**).

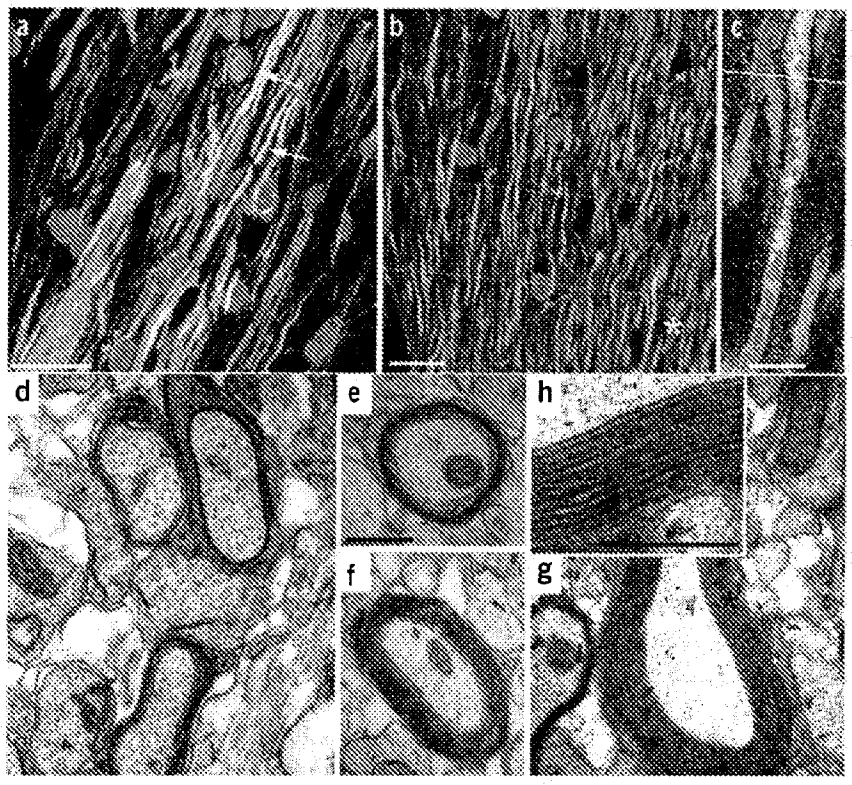

Figure 3 Axonal ensheathment and myelin compaction by engrafted human OPCs. (**a**) Confocal micrograph showing triple immunostain for MBP (red), human nuclear antigen (HNA; blue) and NF (green). All MBP immunostaining is derived from sorted human OPCs, whereas NF$^+$ axons are those of mouse host. Arrows indicate segments of mouse axons ensheathed by human oligodendrocytic MBP. (**b**) Composite of optical sections through corpus callosum of shiverer recipient killed 12 weeks after fetal OPC implantation. (**c**) Higher magnification of area indicated by * in **b**. MBP immunoreactivity (red) surrounds ensheathed axons (green) on both sides. (**d**) Electron micrographs of sagittal section through corpus callosum of adult *shi/shi* homozygote. Shiverer axons typically have single loose wrapping of uncompacted myelin, such that major dense lines do not form. (**e–h**) Representative electron micrographs of 16-week-old homozygous shiverer mice implanted with human OPCs shortly after birth. These images show resident shiverer axons with densely compacted myelin sheaths. **h**, enlargement of area indicated by * in **g**. Major dense lines are visible between myelin lamellae, providing electron microscopic confirmation of myelination by engrafted human OPCs. Scale bar, 20 μm (**a**,**b**), 5 μm (**c**) or 1 μm (**d–h**); **d**, **f**, **g** use bar in e.

that the human progenitors generated myelinating oligodendrocytes in great numbers. Of the recipients scored, 11.9 ± 1.6% (mean ± s.e.m.) of NF$^+$ host callosal axons were surrounded by MBP immunoreactivity (n = 3 mice; three fields scored per animal; Fig. 3a–c). We next used electron microscopy to verify that host axons were fully ensheathed by donor-derived oligodendrocytes, and that the latter generated compact myelin. Because MBP is required to compact consecutive layers of myelin together, its expression is required for the major dense line of mature myelin. Myelin in MBP-deficient shiverer mice did not show more than a few loose wrappings and lacked major dense lines (Fig. 3d), whereas *shi/shi* graft recipients showed compact myelin with major dense lines (Fig. 3e–h). In a sample of MBP$^+$ fields (n = 50) derived from two mice killed 16 weeks after perinatal implant, 7.4% of callosal axons (136 of 1,832 sampled) had donor-derived myelin sheaths, as defined ultrastructurally by their major dense lines. Thus, engrafted fetal human OPCs efficiently differentiated into myelinogenic oligodendrocytes.

Some transplanted fetal OPCs differentiated into GFAP$^+$ astrocytes as early as 4 weeks after implantation. In white-matter regions sampled on the basis of high donor-cell engraftment, 12.7 ± 4.3% of fetal donor-derived cells expressed astrocytic GFAP at 12 weeks, and 10.2 ± 4.4% of donor cells expressed MBP. No heterotopic β-III tubulin- or MAP-2-defined neurons of donor derivation were noted at 4, 8 or 12 weeks after implant (n = 33 total). Nevertheless, 40.3 ± 4.2% of donor cells expressed S100-β, which is expressed by astrocytes and young oligodendrocytes, and nestin was expressed by 47.3 ± 4.2%, suggesting that a large proportion of donor cells persisted as glial progenitors after engraftment. Fetal OPCs were recruited as oligodendrocytes or astrocytes in a context-dependent manner, giving rise to both oligodendrocytes and fibrous astrocytes in the presumptive white matter, but only to GFAP$^+$ astrocytes in the gray matter (Fig. 2f and Supplementary Fig. 2 online).

We next asked whether adult-derived OPCs differed from their fetal counterparts with respect to their dispersal, myelinogenic capacity, or time courses thereof. We implanted two litters of P0 shiverer mice with A2B5-sorted OPCs extracted from adult human subcortical white matter. The mice were killed after 4, 8 or 12 weeks, and their brains were stained for hNA and either MBP or GFAP. Nine of 11 mice were successfully engrafted. The adult OPCs achieved widespread and dense MBP expression by 4 weeks (Fig. 4a–d); at 12 weeks, 39.5 ± 16.3% of adult OPCs expressed MBP. In contrast, none of the hNA$^+$ fetal donor OPCs expressed MBP 4 weeks after engraftment, and only 10.2 ± 4.4% did so by 12 weeks ($P < 0.001$ by two-tailed t-test comparing the proportion of MBP$^+$ cells in fetal and adult-derived grafts; Fig. 4a–c). These results indicate that engrafted adult OPCs were at least four times more likely to become oligodendrocytes and develop myelin than their fetal counterparts. Essentially no adult OPCs became astrocytes in the recipient white matter (none developed GFAP expression), whereas 12.7 ± 4.3% of fetal OPCs did so by 12 weeks. Thus, whereas nominally oligodendrocytic progenitors derived from the fetal brain acted as glial progenitors, adult OPCs behaved in a more restricted manner, largely generating either myelinogenic oligodendrocytes or persistent progenitors in recipient white matter. The more rapid myelination by adult OPCs was reflected ultrastructurally, as the major dense lines of compact myelin were readily evident in mice 6 weeks after implantation with adult OPCs at birth (Fig. 4e). No such evidence of myelin compaction was noted in mice implanted with fetal OPCs until 12–16 weeks postnatally.

Despite the apparent competitive advantage of adult OPCs, substantially more fetal than adult donor cells became engrafted in the recipient brains (Fig. 4f). At the midline of the corpus callosum, the region of maximal engraftment, we scored 1,123 ± 205.6 hNA$^+$ fetal donor cells/mm^2. Of these, 117 ± 43.7 were MBP$^+$, and 9.8 ± 3.1% of fetal donor cells dif-

ferentiated into myelinating oligodendrocytes by 12 weeks. In contrast, only 244 ± 182.1 donor cells/mm^2 were noted in the callosal midline of shiverer mice implanted with adult OPCs. Yet 81 ± 59.7, or 38.9 ± 12.9%, of these cells had developed into MBP$^+$ oligodendrocytes by 12 weeks ($P < 0.001$ by two-tailed t-test comparing the proportion of MBP$^+$ cells in fetal and adult grafts; **Fig. 4g**). In addition, whereas 12.7 ± 4.3% of fetal donor cells matured to express GFAP, no adult donor cells gave rise to GFAP$^+$ astrocytes, again suggesting a stronger bias toward the oligodendrocytic phenotype by the adult progenitors. Thus, besides maturing more quickly than fetal OPCs, adult OPCs gave rise to oligodendrocytes in much higher proportions than their fetal counterparts.

To assess whether adult and fetal OPCs differ in the extent to which they ensheath axons, we scored the numbers of axons myelinated by each donor OPC, as defined by confocal-verified MBP$^+$ wrapping of NF$^+$ axons. These absolute values were then expressed as ratios to total number of donor cells and to donor-derived MBP$^+$ oligodendrocytes per field. When assessed 12 weeks after perinatal graft, adult-derived OPCs ensheathed many more host axons per donor cell than their fetal counterparts, an effect that persisted even after we limited our analysis to the number of ensheathed axons per MBP$^+$ donor cell (**Fig. 4h**). In each case, the difference between fetal and adult donor ensheathment efficiency was significant by Mann-Whitney analysis ($P < 0.02$). Thus, adult-derived OPCs matured to ensheath more axons per donor cell than their fetal counterparts.

These results indicate that isolates of human OPCs sorted from the highly oligoneogenic, late second-trimester forebrain, as well as from adult subcortical white matter, can broadly myelinate the shiverer mouse brain, a genetic model of perinatal leukodystrophy. When introduced as highly enriched isolates, both fetal and adult-derived OPCs spread widely throughout the presumptive white matter, ensheathed resident mouse axons and formed antigenically and ultrastructurally compact myelin. Donor-derived myelinogenesis was geographically extensive and was observed throughout all white matter regions of the telencephalon. After implantation, the mitotic expansion of the cells slowed over time (**Fig. 1g**), and neither undesired phenotypes nor parenchymal aggregates were generated. Both fetal and adult-derived OPCs were capable of remyelinating mouse axons, and neither generated heterotopic neurons. We also noted some marked differences between fetal and adult-derived OPCs. Whereas fetal OPCs were highly migratory, they myelinated slowly and inefficiently, and cogenerated astrocytes in recipient white matter as readily as they did myelinogenic oligodendrocytes. In contrast, adult OPCs migrated over shorter distances, but myelinated more rapidly and in higher proportions than did their fetal counterparts, with virtually no astrocytic coproduction. On an individual basis, each adult OPC–derived oligodendrocyte ensheathed and myelinated substantially more axons than did its fetal-derived counterparts (**Fig. 4g**).

Together, these observations suggest that isolates of human glial progenitor cells may provide effective cellular substrates for remyelinating the congenitally dysmyelinated or hypomyelinated brain. In practical terms, the choice of stage-defined cell type may be dictated by both the availability of donor material and the specific biology of the disease target. Their differences notwithstanding, fetal and adult-derived human OPC isolates were capable of achieving widespread and efficient myelination of the dysmyelinated brain, suggesting new strategies for the treatment of the congenital leukodystrophies and myelin disorders.

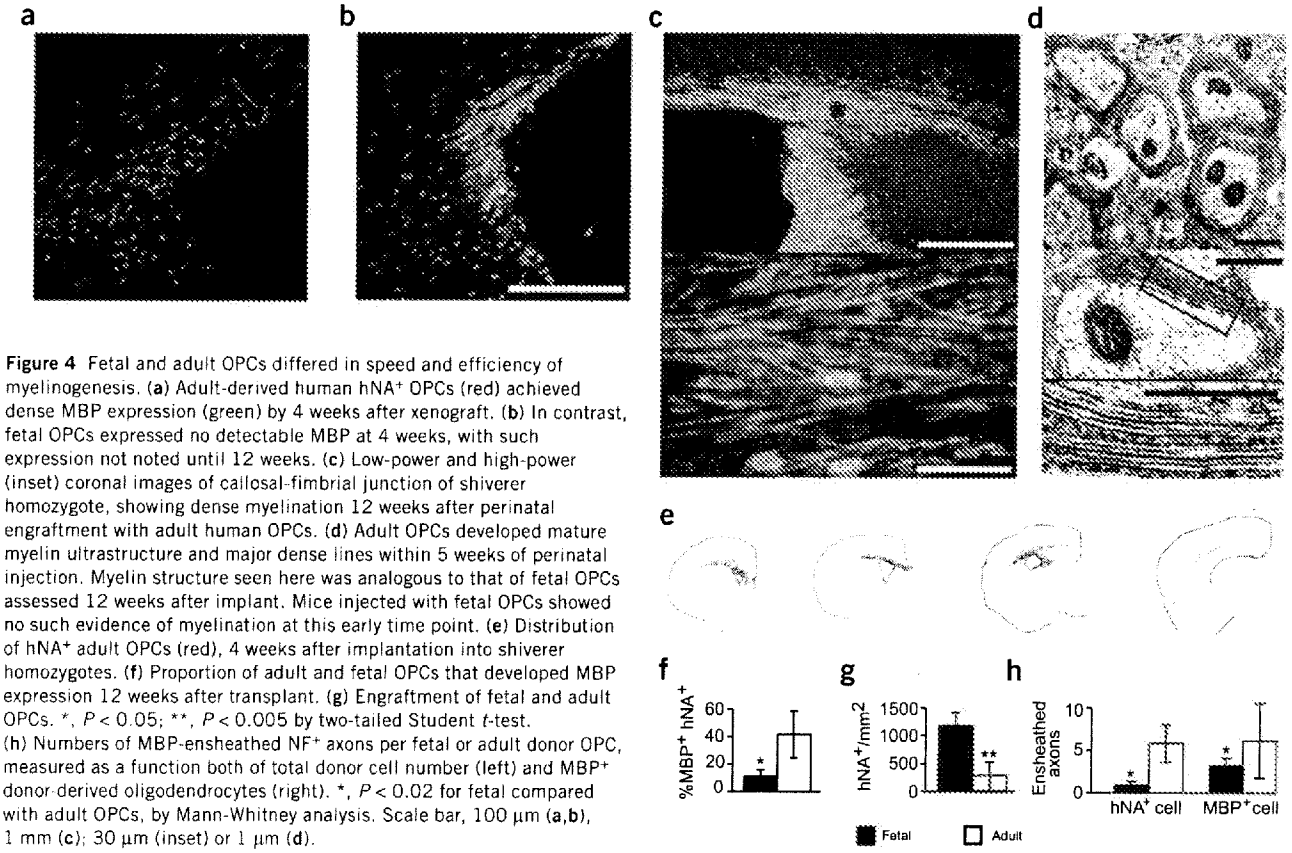

Figure 4 Fetal and adult OPCs differed in speed and efficiency of myelinogenesis. (**a**) Adult-derived human hNA$^+$ OPCs (red) achieved dense MBP expression (green) by 4 weeks after xenograft. (**b**) In contrast, fetal OPCs expressed no detectable MBP at 4 weeks, with such expression not noted until 12 weeks. (**c**) Low-power and high-power (inset) coronal images of callosal-fimbrial junction of shiverer homozygote, showing dense myelination 12 weeks after perinatal engraftment with adult human OPCs. (**d**) Adult OPCs developed mature myelin ultrastructure and major dense lines within 5 weeks of perinatal injection. Myelin structure seen here was analogous to that of fetal OPCs assessed 12 weeks after implant. Mice injected with fetal OPCs showed no such evidence of myelination at this early time point. (**e**) Distribution of hNA$^+$ adult OPCs (red), 4 weeks after implantation into shiverer homozygotes. (**f**) Proportion of adult and fetal OPCs that developed MBP expression 12 weeks after transplant. (**g**) Engraftment of fetal and adult OPCs. *, $P < 0.05$; **, $P < 0.005$ by two-tailed Student t-test. (**h**) Numbers of MBP-ensheathed NF$^+$ axons per fetal or adult donor OPC, measured as a function both of total donor cell number (left) and MBP$^+$ donor-derived oligodendrocytes (right). *, $P < 0.02$ for fetal compared with adult OPCs, by Mann-Whitney analysis. Scale bar, 100 μm (**a**,**b**), 1 mm (**c**); 30 μm (inset) or 1 μm (**d**).

METHODS

Cells. Fetal OPCs were extracted from 21- to 23-week-old human fetuses obtained at abortion. The forebrain ventricular and subventricular zones were dissected free and chilled on ice. The minced samples were dissociated using papain and DNase as described[20,21], always within 3 h of extraction, and maintained overnight in DMEM/F12/N1 with 20 ng/ml fibroblast growth factor. Adult-derived OPCs were collected from subcortical white matter samples obtained at surgery, as described[12,13]. The eight adult tissue samples used were derived largely from patients undergoing temporal lobe resection for medication-refractory epilepsy. No tissues were accepted from patients with known neoplastic disease. Both fetal and adult samples were obtained with consent, using protocols approved by the institutional review boards of Cornell–New York Presbyterian Hospital, and the Albert Einstein College of Medicine and Jacobi Hospital.

Sorting. The day after dissociation, cells from fetal samples were incubated in a 1:1 ratio with monoclonal antibody A2B5 supernatant (clone 105, American Type Culture Collection) for 30 min, then washed and labeled with fluorophore- or microbead-tagged rat antibody to mouse IgM (Miltenyi Biotec). In some instances, two-channel FACS was used to define the proportions and homogeneity of A2B5- and PSA-NCAM-defined subpopulations, using a FACSVantage SE/Turbo (Becton Dickinson) as described[13,21]. For preparative sorting before transplantation, $A2B5^+$ cells were prepared by magnetic separation (Miltenyi Biotec) according to the manufacturer's protocol. The bound cells were eluted and incubated with mouse antibody to PSA-NCAM (1:25; PharMingen) for 30 min, then with phycoerythrin-tagged secondary antibody (1:200). The $PSA\text{-}NCAM^+$ population was then removed by FACS, leaving a highly enriched pool of $A2B5^+PSA\text{-}NCAM^-$ cells. This PSA-NCAM immunodepletion step was omitted for adult samples, which were sorted on the basis of A2B5 only[12,20]. After sorting, both fetal and adult cells were maintained for 1–7 d in DMEM/F12/N1 with 20 ng/ml basic fibroblast growth factor (20 ng/ml) until implantation.

Transplantation and tagging. Homozygous shiverer mice were bred in our colony. Within 1 d of birth, pups were cryoanesthetized for cell delivery. Donor cells (1×10^5) in 2 μl of HBSS were injected through a pulled glass pipette and inserted through the skull into the presumptive corpus callosum. Transplants were directed to the corpus callosum at a depth of 1.0–1.2 mm, depending on the weight of the pup, which varied from 1.0 to 1.5 g. Pups were killed 4, 8, 12 or 16 weeks thereafter. For some experiments, recipient mice were injected for with bromodeoxyuridine (BrdU; 100 μg/g as a 1.5 mg/100 μl solution) every 12 hours 2 d before killing.

Immunohistochemistry. Transplanted cells were identified using antibody 1281 to human nuclei (Chemicon), monoclonal antibody 91 to cyclic nucleotide phosphodiesterase (CNP) protein (Sternberger and Meyer), rabbit antibody to S-100 (Sigma), rabbit antibody to human nestin (gift of H. Okano, Keio University), Sternberger monoclonal antibody 311 to NF, Sternberger monoclonal antibody 21 to human GFAP, rat antibody to BrdU (Harlan) and either Sternberger monoclonal antibody 94 to MBP or rat antibody 7349 to MBP (Abcam), all as described[7,12,20–22].

Confocal and electron microscopy. Confocal imaging was done using an Olympus Fluoview mated to an IX70 inverted microscope, as described[23]. Argon laser lines were used to achieve three-channel immunofluorescence detection of fluorescein-, Texas red– and Cy5-tagged antibodies; the latter was then pseudocolored blue for presentation. For confocal quantification of ensheathment efficacy, shiverer axons were scored as ensheathed when yellow index lines intersected NF^+ axon abutted on each side by MBP immunoreactivity. The proportion of ensheathed axons was defined as the incidence of MBP^+NF^+ axons divided by the total number of NF^+ axons in each field. For electron microscopy, animals were perfused and post-fixed with 4% paraformaldehyde and 0.25% glutaraldehyde in 6% sucrose, then Vibratome-sectioned as alternating thick (400 μm) and thin (100 μm) sections. The latter were immunostained for MBP. Thick sections adjacent to thin sections with MBP expression were then processed in 1% osmium and 1.5% ferricyanide, stained with 1.5% uranyl acetate, embedded in Epon, cut as 100-nm thin sections onto Formvar-coated grids, stained with lead citrate and visualized using a JEOL100 electron microscope[24].

Note: Supplementary information is available on the Nature Medicine website.

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COMPETING INTERESTS STATEMENT

The authors declare competing financial interests (see the Nature Medicine website for details).

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